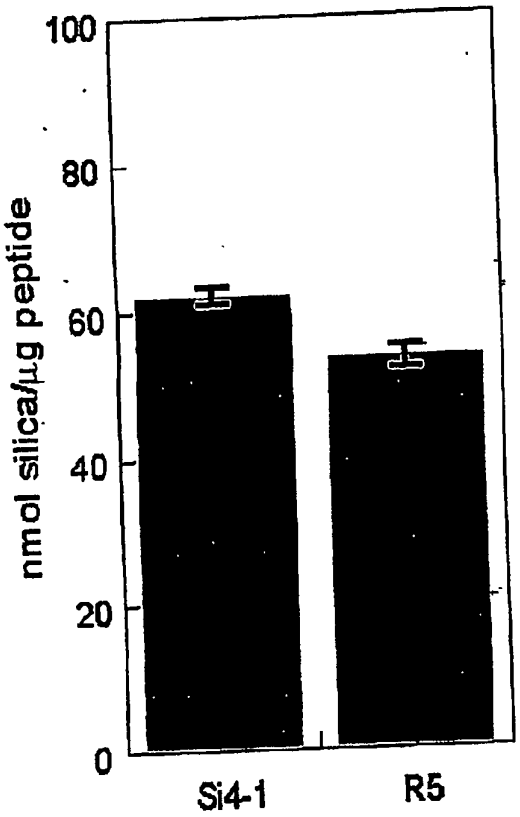
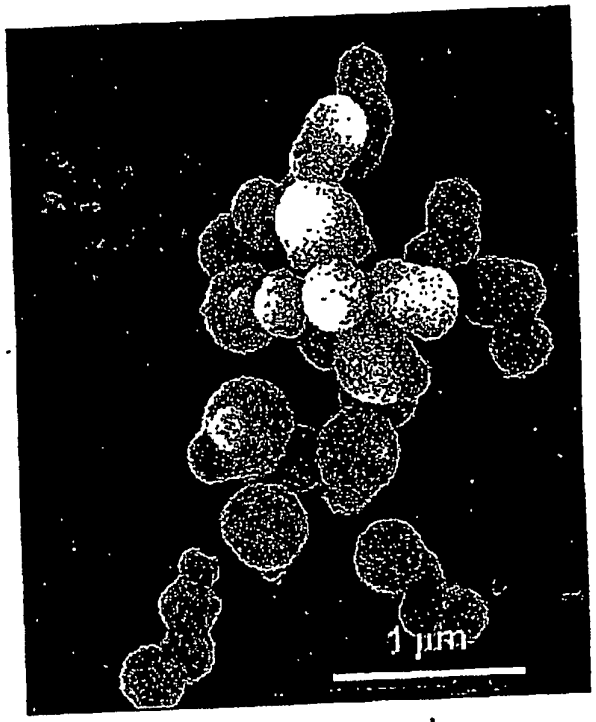
Silica Precipitation Activity of Si4-1 Peptide





SEM Micrograph

FIG. 1A

A 12 amino acid synthesized peptide based on the sequence displayed by phage clone 4-1 is also able precipitate silica similar to the original phage clone.

WO 03/078451
1/14
Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07617
10/507028

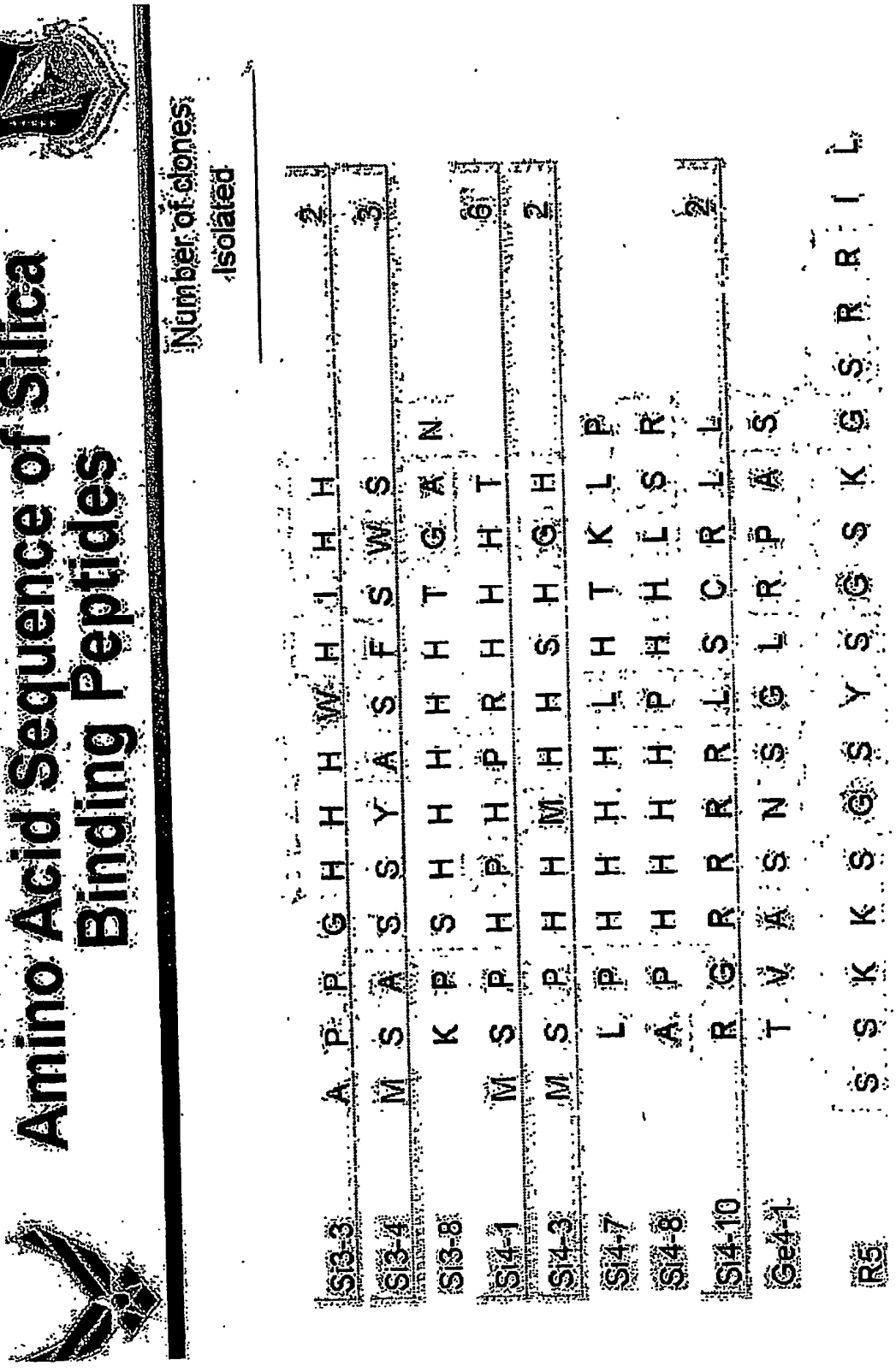

Amino Acid Sequence of Silica Binding Peptides
Number of clones isolated
S3-3 A P P G H H H W H 1 H H 2
S3-4 M S A S S Y A S F S W S 3
S3-8 K P S H H H H H T G A N 6
S4-1 M S P H P H P R H H H T 2
S4-3 M S P H H M H H S H G H
S4-7 L P H H H H L H T K L P
S4-8 A P H H H H P H H L S R 2
S4-10 R G R R R R L S C R L L
Ge4-1 T V A S S N S G L R P A S
R5 S S K K S G G S Y S G S K G S R R I L

FIG. 1B

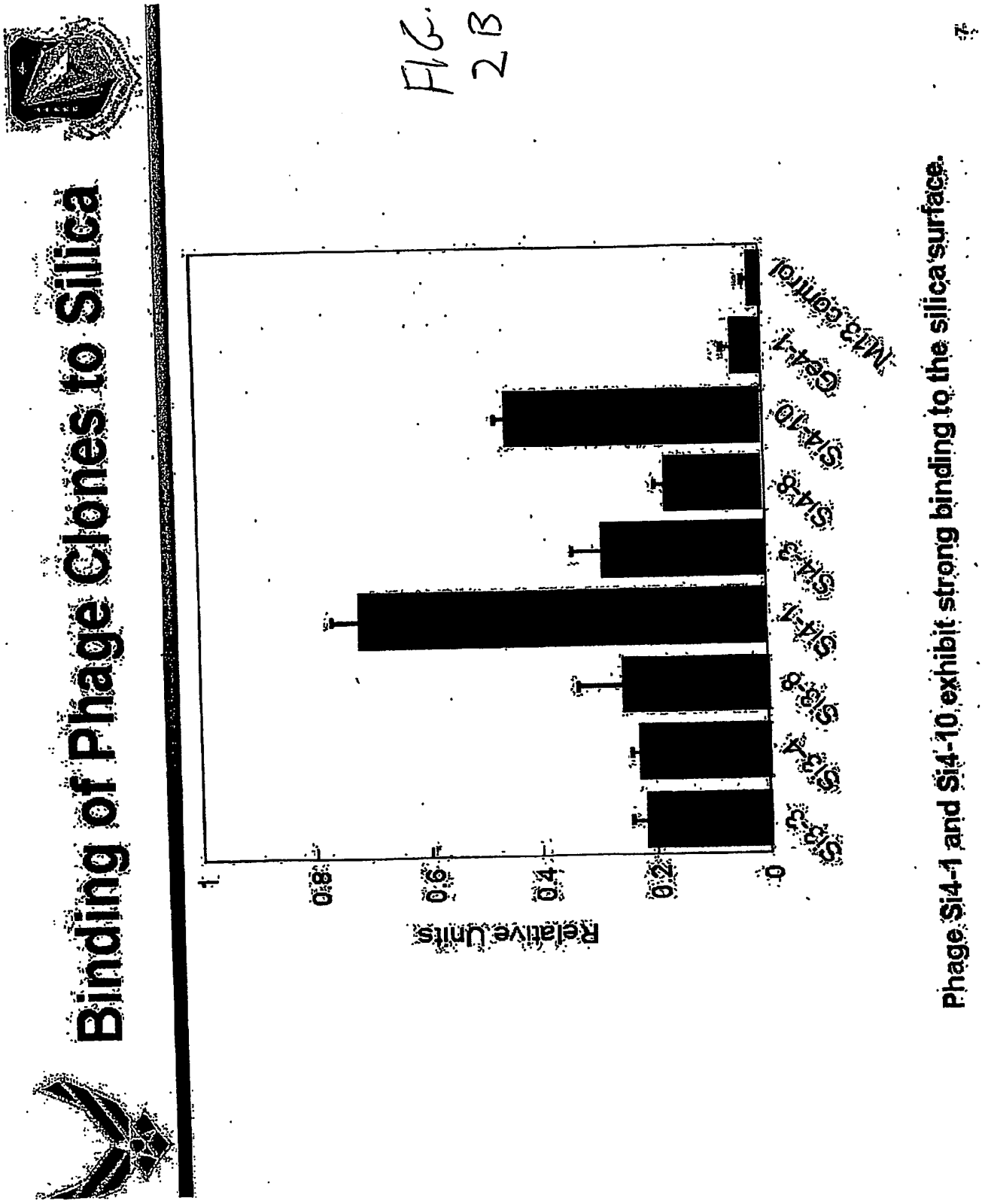
Recognition of Silica by Phage Clones

Phage clones selected against silica exhibit binding to the surface of the silica particles, while a germanium selected clone Ge4-1 shows little or no binding to the silica surface.

FIG. 2A

WO 03/078451
3/14
Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07617
10/507028

FIG. 2B



Phage Si4-1 and Si4-10 exhibit strong binding to the silica surface.

Silica Precipitation Activity of Phage Clones

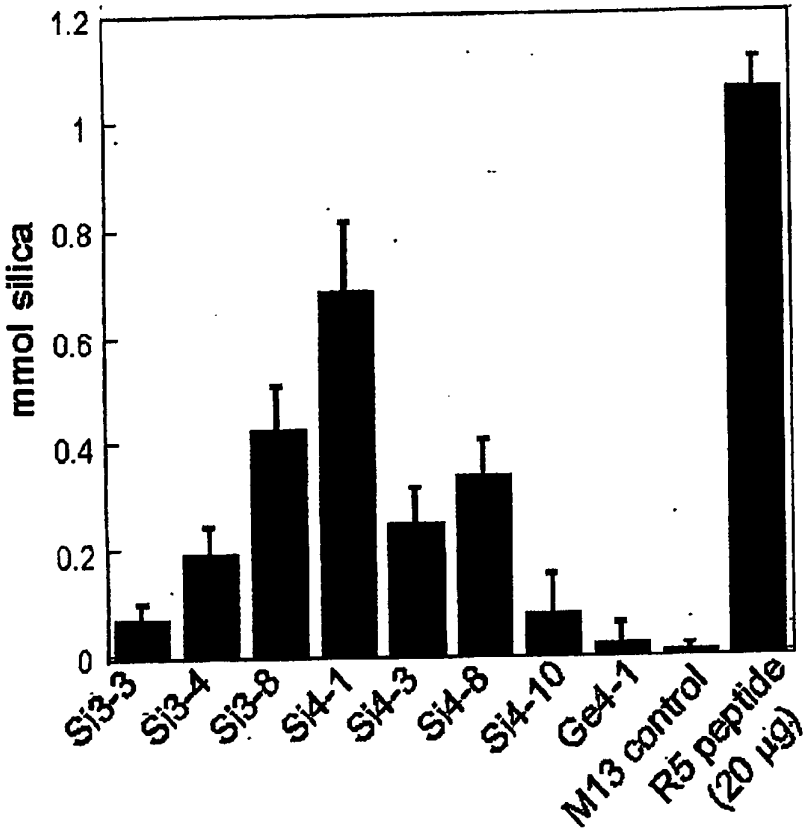
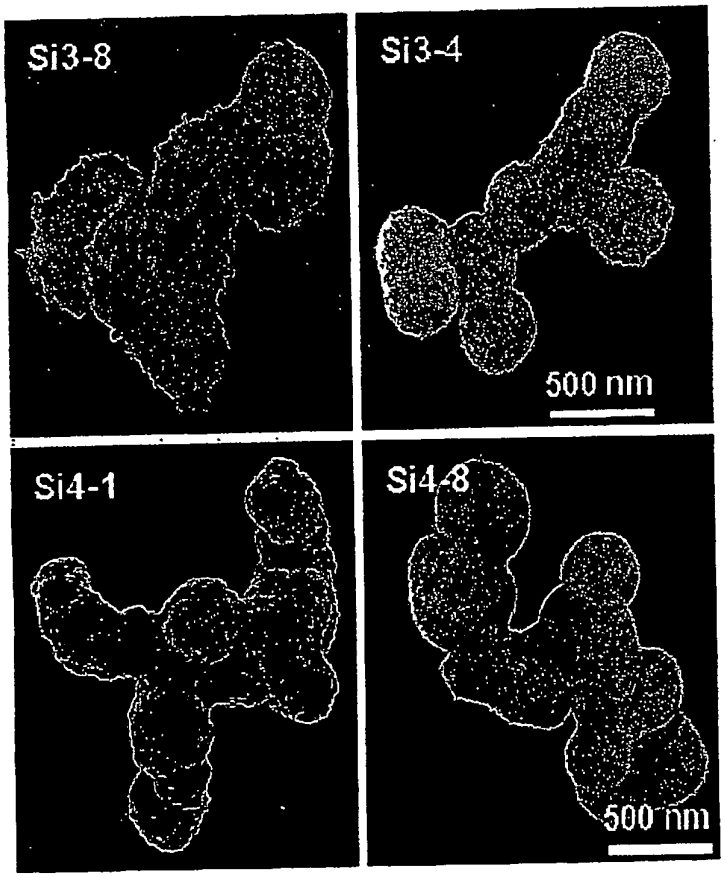


Fig. 3

Silica selected phage clones exhibit silica precipitation activity but to varying levels. Clones Si3-3, Si4-10 or germanium selected clone Ge4-1 exhibit little or no silica precipitation activity.

WO 03/078451
5/14
Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07613
10/507028

SEM Micrographs of Silica Precipitated by Phage Clones



Silica particles fuse to form a network. The diameter of single particles ranges between 200-400 nm

FIG. 4 A

WO 03/078451
6/14
Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07617
10/507028

Structural Analysis of Phage Precipitated Silica

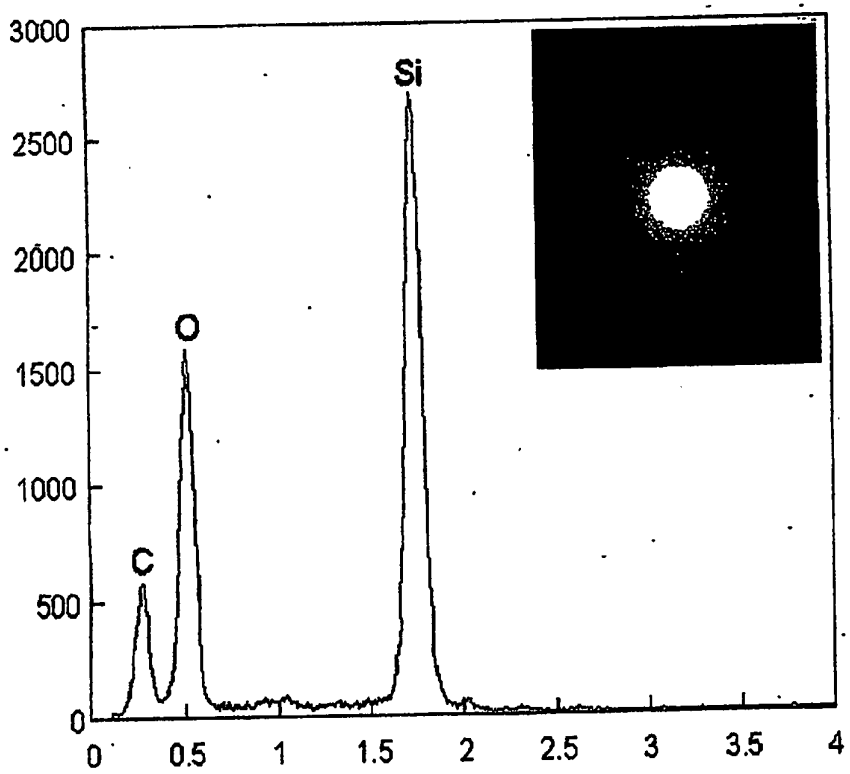
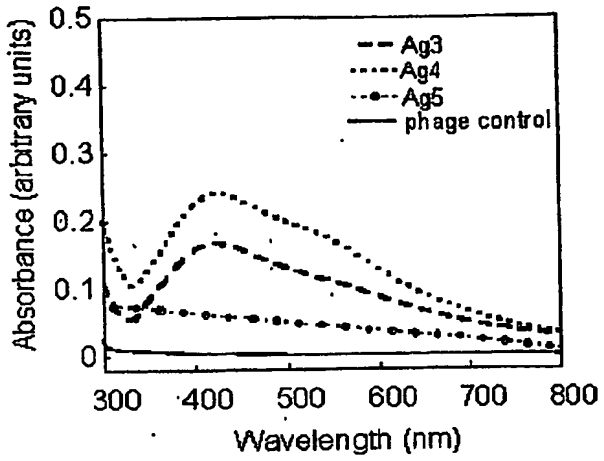


FIG. 4B

The EDX spectrum indicates high silica and oxygen content, the carbon signal is caused either by the peptide or the carbon coated grids used for TEM analysis. The electron diffraction pattern indicates the amorphous nature of the silica precipitate.

WO 03/078451
7/14
Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07617
10/507028

A


Absorbance (arbitrary units)
0.5
0.4
0.3
0.2
0.1
0
300
400
500
600
700
800
Wavelength (nm)
Ag3
Ag4
Ag5
phage control

B

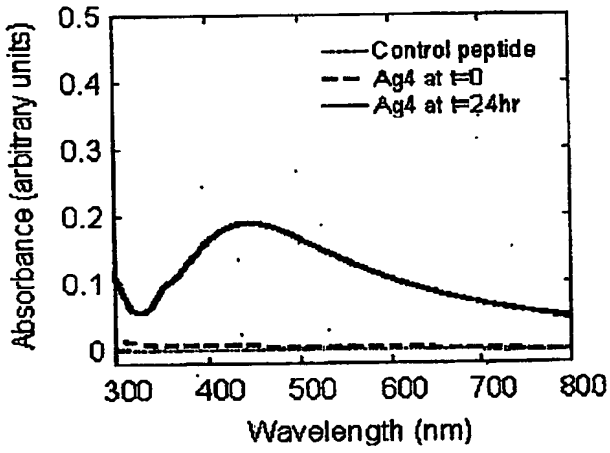

Absorbance (arbitrary units)
0.5
0.4
0.3
0.2
0.1
0
300
400
500
600
700
800
Wavelength (nm)
Control peptide
Ag4 at t=0
Ag4 at t=24hr

Figure 5

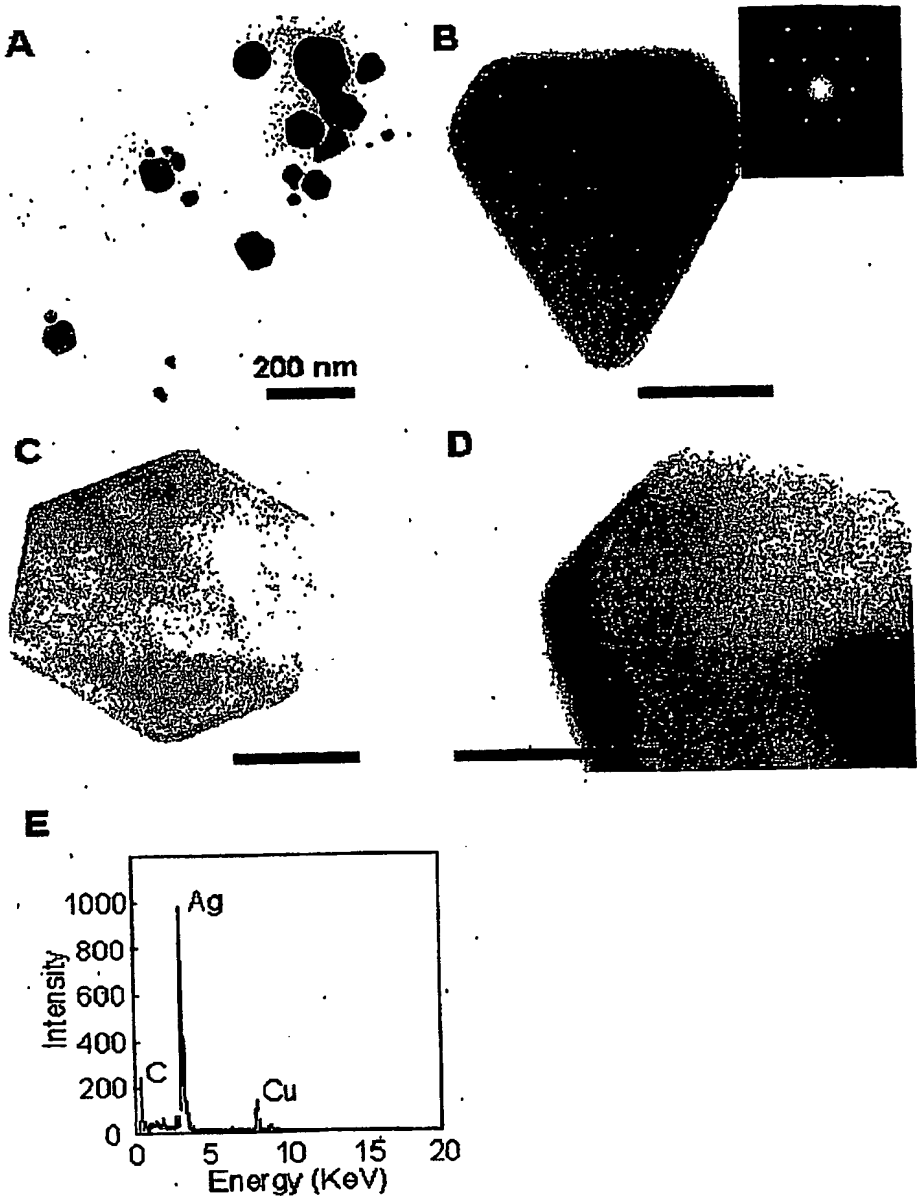


Figure 6

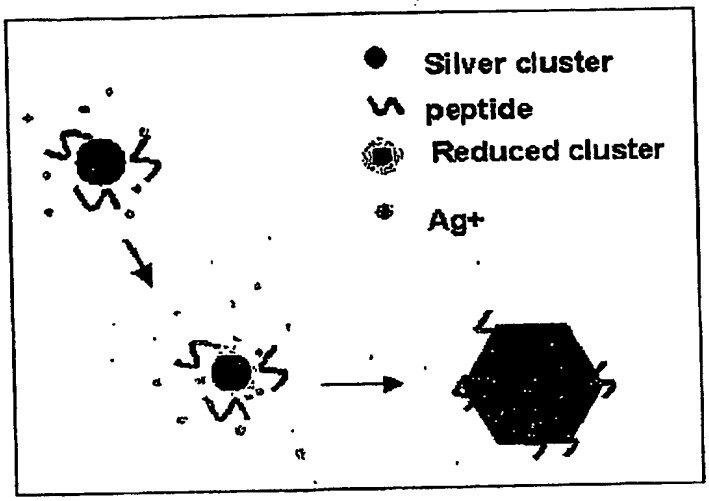


Figure 7

Rec'd PCT/PTO 03 SEP 2004

PCT/US03/07617

10/507028

A

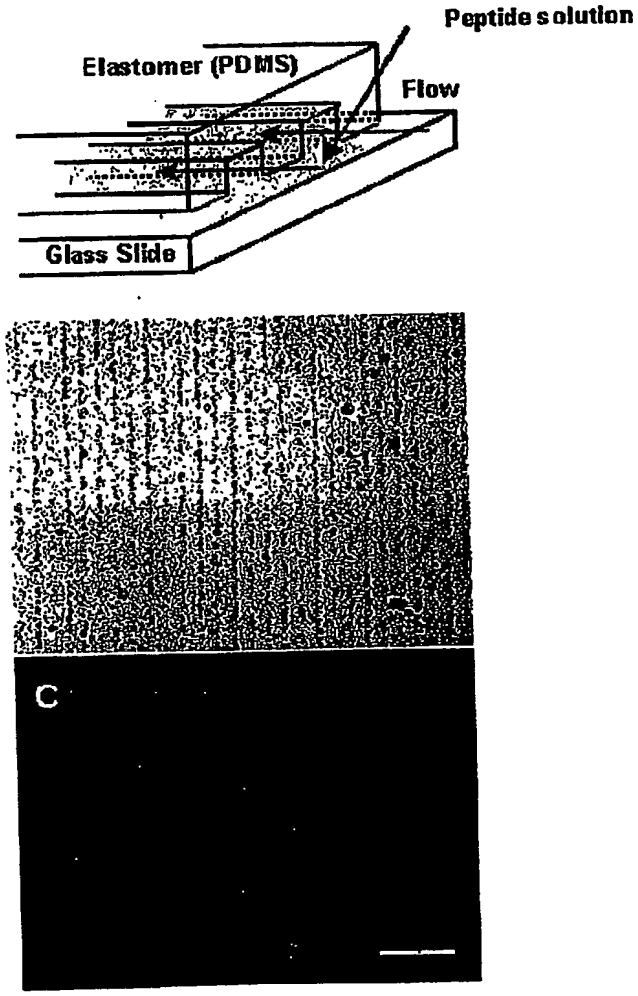


Figure 8

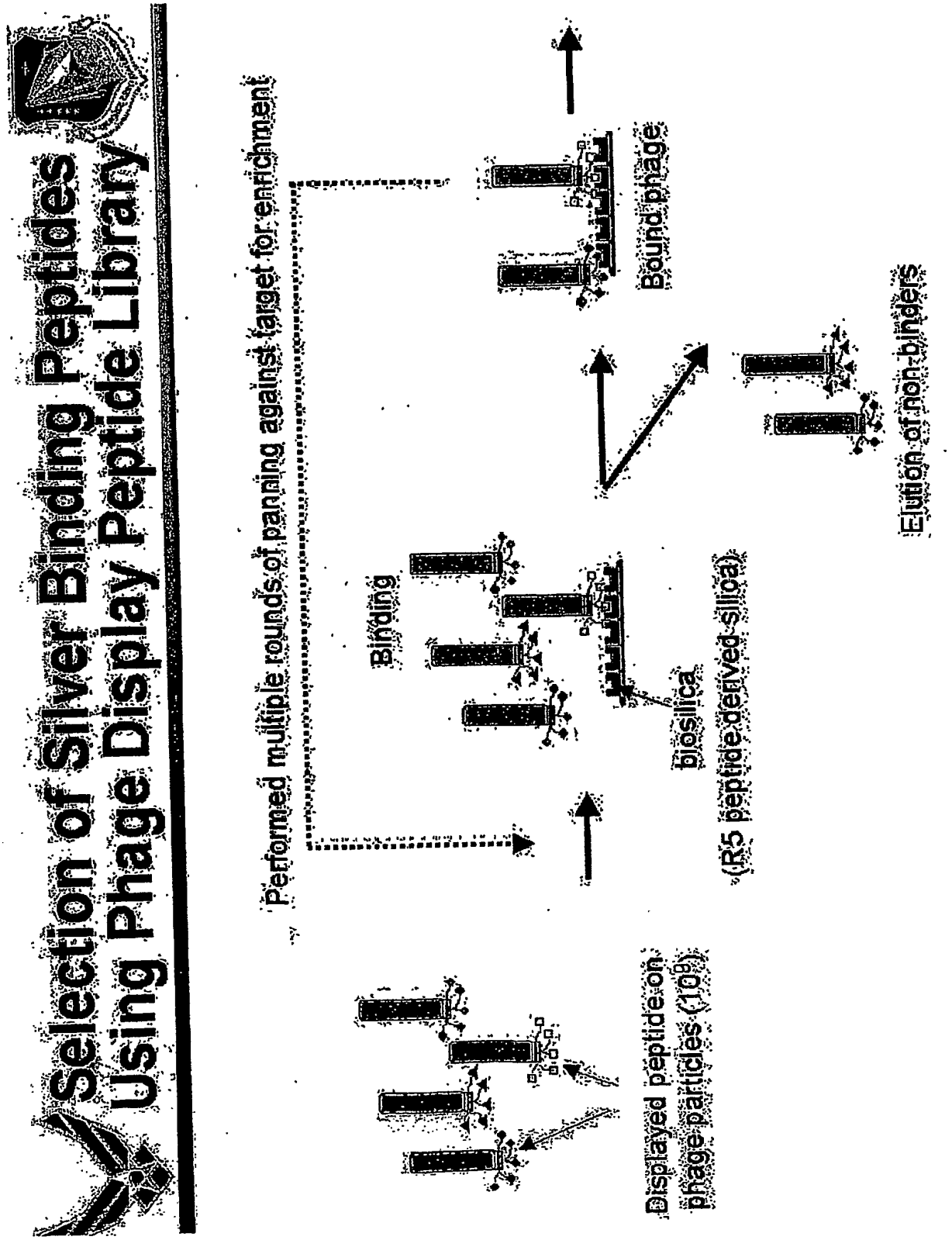

Selection of Silver Binding Peptides
Using Phage Display Peptide Library
Performed multiple rounds of panning against target for enrichment
Displayed peptide on phage particles (10⁸)
Binding
biosilica
(R5 peptide-derived-silica)
Bound phage
Elution of non-binders

FIG. 9

Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07617
10/507028

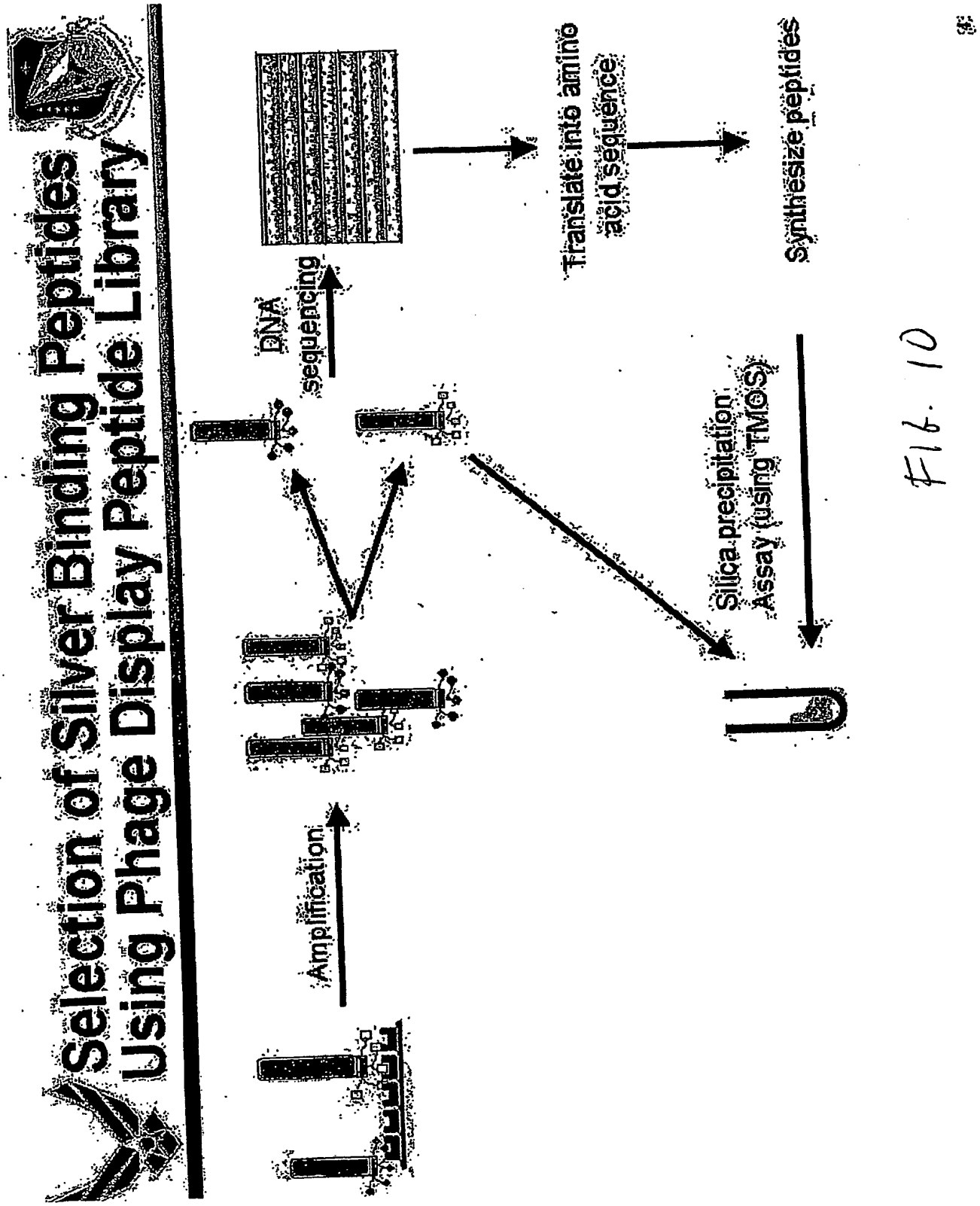


Fig. 10

Characteristics of the Selected Phage Clones

Phage Clone	Histidine Residues	Hydroxyl-containing Residues	pI	Silica Precipitating Activity (nmoles) [rank]
Si3-3	6	0	7.24	60 [8]
Si3-4	0	6	5.27	187 [6]
Si3-8	5	2	8.78	420 [3]
Si4-1	5	2	9.57	680 [1]
Si4-3	6	2	7.01	240 [5]
Si4-7	5	1	8.78	500 [2]
Si4-8	6	1	9.83	334 [4]
Si4-10	0	1	12.3	73 [7]

Based on the amino acid sequence information, peptides that have hydroxyl -containing amino acids and a high pI are essential for silica precipitating activity.

Fig. 11.

WO 03/078451
14/14
Rec'd PCT/PTO 08 SEP 2004
PCT/US03/07617
10/507028